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Washington, D. C. April, 1926

CHEMISTRY AND ANALYSIS OF THE PERMITTED COAL-TAR FOOD DYES

By

JOSEPH A. AMBLER, Chemist, W. F. CLARKE, Assistant Chemist, O. L. EVENSON, Assistant Chemist, H. WALES, Associate Chemist, Color Laboratory, Bureau of Chemistry

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INTRODUCTION

In 1912 Hesse (*25*)[1] described the work on aniline dyes used in foods which was done in the Bureau of Chemistry after the passage of the Federal food and drugs act in 1907, and compiled the literature on the subject then available. At that time only 7 coal-tar dyes were permitted for certification; during the next 10 years 4 new dyes were added, so that on January 1, 1926, 11 coal-tar dyes were recognized as harmless and considered permissible for use in foods (Food Inspection Decisions 164, 175, 180, 184). Since the certification plan, discussed in detail by Hesse (*25*), was devised it has been found necessary from time to time to change certain details of the procedure and of the methods of analysis. Several of these changes have been given in food inspection decisions (Nos. 117, 129, and 159)

[1] Italic numbers in parenthesis refer to "Literature cited," page 35.

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and in scientific publications (*45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55*); others have been announced only through official correspondence. The present procedure for certification is outlined in United States Department of Agriculture Miscellaneous Circular 52; the chemistry of the permitted coal-tar food colors and the revised methods of their analysis are fully described in the following pages.

CHEMISTRY OF THE PERMITTED COAL-TAR FOOD DYES

In the preparation and purification of dyes to be certified care must be taken not only in the final steps of their manufacture, but also in the synthesis or purification of their intermediates. Many intermediates are commercial products containing relatively large proportions of isomeric and subsidiary compounds, which will produce isomeric and subsidiary colors during the preparation of the dye. Some of these impurities are harmful, and all are undesirable adulterants. Purification of adulterated intermediates is not worth trying. The only safe method is to use intermediates of the best possible quality. The Bureau of Chemistry has refused to approve for certification several batches of dyes because they contained foreign coloring matter. These rejections would not have been made if intermediates of suitable quality had been used.

Several of the permitted dyes can be prepared by more than one method. The method of manufacture is immaterial, so long as the final product meets the proper specifications. The incorporation in the foundation affidavit (*70*) of an outline of the method of manufacture, with the names of the intermediates used and the process employed, gives helpful information to the analysts who seek to verify the identity of the dye. If the firm submitting the foundation affidavit does not manufacture but simply purifies commercial dyes, an outline of the process of purification is of material aid in the examination of the dye. Such information is not required, but it is very helpful. When incorporated in the foundation affidavit it is held in strictest confidence by the Bureau of Chemistry.

With the exception of yellow A B and yellow O B, the permitted coal-tar dyes are hygroscopic. Consequently, when a dye is being prepared with a view to requesting its certification, after the batch has been finely ground and mixed to uniform composition, it should be dried only to the condition in which it would neither lose nor gain unduly large quantities of moisture if exposed for brief periods to an atmosphere of normal humidity at the place of preparation.

PONCEAU 3R

Ponceau 3R ($C_{19}H_{16}N_2O_7S_2Na_2$), a monazo dye (*68*), is made by diazotizing crude ψ-cumidine and coupling the product with "R" acid (β-naphthol-3-6-disulphonic acid). Because crude ψ-cumidine is a mixture, it is necessary to define the grade which may be used in the manufacture of the dye. The product from pure ψ-cumidine, known as ponceau 4R, has such a strong bluish-red color that it has proved unsatisfactory. The shade wanted is produced from crude ψ-cumidine having a boiling point of 220° C. or higher. The affidavit (*70*) accompanying a sample of ponceau 3R to be certified must contain a statement of the boiling point found by testing the crude ψ-cumidine used for the batch concerned.

Care is necessary also in selecting the "R" acid. This acid should be free from the isomeric "G" acid (β-naphthol-6–8-disulphonic acid) and from the lower sulphonated Schaeffer's acid (β-naphthol-6-sulphonic acid). The presence of either of these impurities in the intermediate makes it practically impossible to remove the resulting subsidiary dye from the finished product.

Unless sufficient care is used in the coupling and other procedures a batch will develop a very unpleasant odor, which persists when the dye is used in coloring food. This odor is probably the result of side reactions, which may occur during the diazotization and coupling of the ψ-cumidine, giving rise to phenolic substances or other degradation products.

Ponceau 3R is a dark-red powder, which dissolves in water, forming a cherry-red solution. It is only slightly soluble in alcohol. The addition of hydrochloric acid to an aqueous solution does not change the color, but the addition of sodium hydroxide precipitates from it a yellow powder. Ponceau 3R dissolves in concentrated sulphuric acid to form a cherry-red solution, which remains unchanged in color upon dilution with water.

In 1878 the following patents for the preparation of ponceau 3R were issued: German patent 3,229 (*56*); British patent 1,715 (*56*); French patent 124,811 (*56*). In 1881 United States patent 251,163 (*56*) was issued.

The analytical data required in foundation and supplemental affidavits (*70*), the methods of analysis, and the purity specifications are listed in Table 1.

TABLE 1.—*Specifications for ponceau 3R*

Component	Maximum tolerance (ratio to pure coal-tar dye)	Minimum tolerance	Method of analysis
	Per cent	*Per cent*	*Page*
Moisture	12.0		14.
Insoluble matter:			
Total	.3		15.
Nonvolatile	.3		15.
Sodium chloride	5.0		15, 16.
Sodium sulphate	.5		17.
Sulphated ash	[1]		18.
Sodium in color	Theoretical.		18.
Heavy metals	None.		19.
Calcium	0.3		19.
Magnesium	.3		19.
Aluminium	.1		19.
Iron	.1		19.
Arsenic as As_2O_3	.00014		22, 23.
Ether extractives:			
Neutral	[1]		23.
Alkaline	[1]		23.
Acid	[1]		24.
Total	.2		24.
Sulphur in color	Theoretical.		24.
Color acid		74.7	25.
Pure coal-tar dye		82	26.
Lower sulphonated dyes	5.0		29.
Boiling point of crude ψ-cumidine, or of ψ-cumidine by reduction of dye.		[2] 220	29, 30.

[1] Although no limit is here defined, a statement of the percentage found by analysis must be incorporated in the foundation and supplemental affidavits (*70*).

[2] Degrees centigrade.

AMARANTH

Amaranth ($C_{20}H_{11}N_2O_{10}S_3Na_3$), a monazo dye, is made by diazotizing naphthionic acid and coupling the product with "R" acid (*2*, *31*). Very pure naphthionic acid is easily obtainable, and no difficulty traceable to this intermediate has been encountered. It is most important, however, that the "R" acid used be practically free from the isomeric "G" acid and from the lower sulphonated Schaeffer's acid. These couple with naphthionic acid to produce new coccine and fast red E, respectively, which are objectionable in food colors.

Amaranth is very soluble in water, but is readily precipitated from its water solution by salting out with sodium chloride. For batches intended for certification great care and experience are needed in this operation; otherwise too much salt may be occluded with the dye, necessitating the reworking of the batch to bring the percentage salt content within the limits specified.

Amaranth is a reddish-brown powder, which dissolves in water to form a magenta-red solution. It is sparingly soluble in alcohol. When hydrochloric acid is added to an aqueous solution the color is unchanged. The addition of sodium hydroxide to the solution intensifies the color. In concentrated sulphuric acid it forms a violet solution, which changes through a bluish-violet to a magenta-red on dilution with water.

In 1878 the following patents for the preparation of amaranth were issued: German patents 3,229 (*56*) and 5,411 (*4*); British patents 786 (*4*) and 1,715 (*56*); French patents 123,148 (*4*) and 124,811 (*56*); and United States patent 204,799 (*4*).

The analytical data required in foundation and supplemental affidavits (*70*), as well as the purity specifications to which the dye must conform and the methods used in analyzing the dye, are listed in Table 2.

TABLE 2.—*Specifications for amaranth*

Component	Maximum tolerance (ratio to pure coal-tar dye)	Minimum tolerance	Method of analysis
	Per cent	*Per cent*	*Page*
Moisture	15.0		14.
Insoluble matter:			
Total	.4		15.
Nonvolatile	.2		15.
Sodium chloride	4.0		15, 16.
Sodium sulphate	.5		17.
Sulphated ash	(1)		18.
Sodium in color	Theoretical.		18.
Heavy metals	None.		19.
Calcium	0.3		19.
Magnesium	.3		19.
Aluminium	.1		19.
Iron	.1		19.
Arsenic as As_2O_3	.00014		21, 22, 23.
Ether extractives:			
Neutral	(1)		23.
Alkaline	(1)		23.
Acid	(1)		24.
Total	.2		24.
Sulphur in color	Theoretical.		24.
Color acid		73	25.
Pure coal-tar dye		82	26.
Lower sulphonated dyes as fast red E	4.0		28.
Isomeric dyes as new coccine			30.

[1] Although no limit is here defined, a statement of the percentage found by analysis must be incorporated in the foundation and supplemental affidavits (*70*).

ERYTHROSINE

Erythrosine ($C_{20}H_8O_6I_4Na_2$), a xanthene dye, is the sodium salt of tetra-iodofluorescein, containing the equivalent of 1 molecule of water firmly bound in the molecule (*22, 73*). It seems to be impossible to remove this molecule of water without destroying the coloring matter itself. Erythrosine is prepared by iodinating fluorescein, a dye made by condensing phthalic anhydride with resorcinol, usually aided by zinc chloride or sulphuric acid (*12, 58, 61, 62*). The procedure must be such that 4 atoms of iodine are introduced into the molecule. This step is the one which requires the most attention in the manufacture. The conditions under which this may be done successfully can be determined only by experimentation.

Erythrosine is a brown powder, which dissolves in water to form a cherry-red solution that shows no fluorescence in ordinary light. When hydrochloric acid is added to its solution, a yellowish-brown precipitate of the free color acid is produced. The addition of sodium hydroxide produces a red precipitate soluble in excess of the reagent. The addition of concentrated sulphuric acid dissolves erythrosine, forming a brownish-yellow solution, which evolves iodine on heating and from which the brownish-yellow color acid is precipitated on diluting with water. The color acid is insoluble in water and is precipitated from solutions of the dye, even by very weak acids. For this reason erythrosine is not suitable for use in coloring food products and beverages which have acid reactions.

In 1899 German patent 108,838 (*67*) for the preparation of erythrosine was issued.

The analytical data required in the foundation and supplemental affidavits (*70*), the methods by which the dye may be analyzed, and the purity specifications are listed in Table 3.

TABLE 3.—*Specifications for erythrosine*

Component	Maximum tolerance (ratio to pure coal-tar dye)	Minimum tolerance	Method of analysis
	Per cent	*Per cent*	*Page*
Moisture	15.0		14.
Insoluble matter:			
Total	.2		15.
Nonvolatile	.1		15.
Sodium chloride	1.0		16.
Sodium sulphate	1.0		17.
Sulphated ash	[1]		18.
Sodium in color	Theoretical.		18.
Heavy metals	None.		19.
Calcium	0.3		19.
Magnesium	.3		19.
Aluminium	.1		19.
Iron	.1		19.
Arsenic as As_2O_3	.00014		22, 23.
Ether extractives:			
Neutral	[1]		24.
Alkaline	[1]		24.
Total	.1		24.
Color acid		76.30	26.
Pure coal-tar dye		82.00	26.
Sodium iodide	.4		30.
Iodine organically combined		[2] 55.70	30.
Total halogens		[2] 56.63	31.
Sodium carbonate	.4		31.

[1] Although no limit is here defined, a statement of the percentage found by analysis must be incorporated in the foundation and supplemental affidavits (*70*).
[2] Ratio to pure coal-tar dye.

ORANGE I

Orange I ($C_{16}H_{11}N_2O_4SNa$), a monazo dye (*41, 42, 60, 74, 75*), is made by coupling diazotized para-sulphanilic acid with α-naphthol. Sufficiently pure para-sulphanilic acid is readily obtainable. The purity of the α-naphthol used, however, is of the utmost importance. The percentage of β-naphthol present in this intermediate should be as low as practicable, as β-naphthol coupled with para-sulphanilic acid produces orange II, which is so toxic that it is extremely objectionable and is tolerated only in small quantity in the final product if the orange I is to be used for coloring foods or beverages. It is safer to use α-naphthol made from α-nitronaphthalene than that made from α-naphthalene-sulphonic acid. Even when α-naphthol from α-nitronaphthalene is used, care must be taken to provide that the α-naphthylamine formed in the process be transformed as completely as possible into the naphthol. To the extent that this is incomplete the objectionable insoluble coloring matter sulphobenzeneazo-α-naphthylamine will be present in the final product.

Because calcium forms an insoluble salt with orange I, only lime-free water should be used as a solvent in the final stages of the process of manufacture and in the purification of the dye, if it is to be of certified grade. It has been shown that even small quantities of the calcium salt cause the solution of the dye to "set" to a stiff, opaque, jellylike mass.

Foundation and supplemental affidavits (*70*) for this dye should contain analytical data on the determinations listed in Table 4, which also enumerates the methods of analysis and shows the purity specifications.

TABLE 4.—*Specifications for orange I*

Component	Maximum tolerance (ratio to pure coal-tar dye)	Minimum tolerance	Method of analysis
	Per cent	*Per cent*	*Page*
Moisture	10.0		14.
Insoluble matter:			
Total	.3		15.
Nonvolatile	.2		15.
Sodium chloride	3.0		15, 16.
Sodium sulphate	.5		17.
Sulphated ash	(1)		18.
Sodium in color	Theoretical.		18.
Heavy metals	None.		19.
Calcium	0.05		19.
Magnesium	.3		19.
Aluminium	.1		19.
Iron	.1		19.
Arsenic as As_2O_3	.00014		22, 23.
Ether extractives:			
Neutral	(1)		23.
Alkaline	(1)		23.
Acid	(1)		24.
Total	.2		24.
Sulphur in color	Theoretical.		24.
Color acid		76.8	25.
Pure coal-tar dye		82.0	26.
Subsidiary dyes (as orange II)	5.0		33.

[1] Although no limit is here defined, a statement of the percentage found by analysis must be incorporated in the foundation and supplemental affidavits (*70*).

. Orange I, a reddish-brown powder, dissolves in water to form an orange-red solution. Its alcoholic solution is orange. The addition

of hydrochloric acid to an aqueous solution produces a brown precipitate. The addition of sodium hydroxide to an aqueous solution turns the color cherry-red. This dye is particularly sensitive to traces of alkali and should be used only for neutral or weakly acid foods and beverages. It dissolves in concentrated sulphuric acid to form a violet-red solution, from which it is precipitated as a red-brown mass when diluted with water.

NAPHTHOL YELLOW S

Naphthol yellow S ($C_{10}H_4N_2O_8SNa_2$), a nitro dye, is the sodium salt of 2-4-dinitro-α-naphthol-7-sulphonic acid (*15*, *23*, *34*, *37*). It is prepared by the nitration of the tri- or disulphonic acids of α-naphthol or from the nitroso compound of the 2-7-disulphonic acid (*24*). In any case, care must be taken to guard against the production of the unsulphonated subsidiary dye martius yellow, which is one of the most poisonous coal-tar dyes known. More than a very small trace of martius yellow makes the naphthol yellow S unsuitable for use in coloring foods.

Being a nitro compound, naphthol yellow S should be pulverized with extreme caution, owing to the danger of explosion. Experience has shown that it may be satisfactorily ground and pulverized in a ball mill.

Table 5 shows the analytical data which should be incorporated in foundation and supplemental affidavits (*70*) and the methods of analysis to be used, and lists the purity specifications for the dye.

TABLE 5.—*Specifications for naphthol yellow S*

Component	Maximum tolerance (ratio to pure coal-tar dye)	Minimum tolerance	Method of analysis
	Per cent	*Per cent*	*Page*
Moisture	10.0		14.
Insoluble matter:			
Total	.2		15.
Nonvolatile	.1		15.
Sodium chloride	2.0		16.
Sodium sulphate	.2		18.
Sulphated ash	(1)		18.
Sodium in color	Theoretical.		18.
Heavy metals	None.		19.
Calcium	0.2		19.
Magnesium	.2		19.
Aluminium	.1		19.
Iron	.1		19.
Arsenic as As_2O_3	.00014		21, 22, 23.
Ether extractives:			
Neutral	(1)		23.
Alkaline	(1)		23.
Acid	(1)		24.
Total	0.1		24.
Sulphur in color	Theoretical.		24.
Color acid		71.9	26.
Pure coal-tar dye		82.0	26.
Martius yellow	0.03		33.

[1] Although no limit is here defined, a statement of the percentage found by analysis must be incorporated in the foundation and supplemental affidavits (*70*).

Naphthol yellow S is a light yellow or orange-yellow powder, which burns with yellow scintillations. The free acid crystallizes from hydrochloric acid solution in fine needles. The dye is readily soluble in water, producing a yellow solution, the color of which is

not altered by either hydrochloric acid or sodium hydroxide. Potassium hydroxide, however, produces a yellow flocculent precipitate. Naphthol yellow S dissolves in concentrated sulphuric acid to form a yellow solution, which becomes brighter upon dilution with water. When reduced with zinc dust and acetic acid the solution is decolorized, but, upon contact with the air, it is reoxidized, with the production of a salmon color.

In 1879 the following patents for the preparation of naphthol yellow S were issued: German patent 10,785 (*5*); British patent 5,305 (*5*). In 1880 the following were issued: French patent 134,632 (*5*); United States patent 225,108 (*5*). In 1882 German patent 20,716 (*66*) and British patent 5,692 (*40*) were issued. In 1883 United States patent 289,543 (*40*) was issued. In 1887 British patent 11,318 (*39*) was issued.

TARTRAZINE

Tartrazine ($C_{16}H_9N_4O_9S_2Na_3$), a yellow dye, may be classed as an azo dye, or, more properly, as a pyrazolone dye (*3*, *11*, *18*, *30*, *36*, *65*, *73*, *77*). Several methods for its preparation are given in the literature (*32*, *64*, *69*). The intermediates used differ according to the method. The principal methods are by the interaction of phenylhydrazine-*p*-sulphonic acid and dioxytartaric acid and by treating phenylhydrazine-*p*-sulphonic acid with oxalacetic ester, coupling the product with diazotized sulphanilic acid and hydrolyzing the resulting ester with sodium hydroxide solution. It is reported also that it may be produced by coupling diazotized sulphanilic acid with oxalacetic ester, condensing the product so formed with phenylhydrazine-*p*-sulphonic acid and hydrolyzing the resulting ester with sodium hydroxide solution. The first method is the simplest of operation and the one which is generally used. Care must always be taken that either the intermediates are transformed completely in the process of manufacture or the unchanged portions are removed completely by purification of the dye.

Tartrazine, a yellow-orange powder, is soluble in water. The resulting golden-yellow solution is not altered by the addition of hydrochloric acid, but becomes redder when sodium hydroxide is added. The dye dissolves in concentrated sulphuric acid to produce an orange-yellow solution, which becomes yellow when diluted with water. Knecht (*33*) and Meyer (*57*) discuss its application as a dye.

In 1885 the following patents for the preparation of tartrazine were issued: German patent 34,294 (*6*); British patent 9,858 (*6*); French patent 169,964 (*6*); United States patent 324,630 (*6*). In 1887 British patent 8,504 (*10*) was issued. In 1888 German patent application B–8,432 (*10*) was issued. In 1893 British patent 5,693 (*76*) was issued. In 1897 German patent application B–20,084 (*6*) and British patent 765 (*6*) were issued.

Table 6 lists the analytical determinations which should be made upon tartrazine, the methods to be used in analysis, and the purity specifications (*70*).

TABLE 6.—*Specifications for tartrazine*

Component	Maximum tolerance (ratio to pure coal-tar dye)	Minimum tolerance	Method of analysis
	Per cent	*Per cent*	*Page*
Moisture	10.0		14.
Insoluble matter:			
Total	.3		15.
Nonvolatile	.2		15.
Sodium chloride	5.0		15, 16.
Sodium sulphate	.5		17.
Sulphated ash	(1)		18.
Sodium in color	Theoretical.		18.
Heavy metals	None.		19.
Calcium	0.3		19.
Magnesium	.3		19.
Aluminium	.1		19.
Iron	.1		19.
Arsenic as As_2O_3	.00014		21, 22, 23.
Ether extractives:			
Neutral	(1)		23.
Alkaline	(1)		23.
Acid	(1)		24.
Total	.3		24.
Sulphur in color	Theoretical.		24.
Color acid		71.9	25.
Pure coal-tar dye		82.0	26.
Lower sulphonated subsidiary dyes	3.0		28.

[1] Although no limit is here defined, a statement of the percentage found by analysis must be incorporated in the foundation and supplemental affidavits (*70*).

YELLOW A B

Yellow A B ($C_{16}H_{13}N_3$), an oil-soluble monazo dye, is made by coupling diazotized aniline with β-naphthylamine (*8, 19, 20, 21, 38*). Both the unchanged portions of the intermediates and the decomposition products of the diazotized aniline must be removed completely from the dye by means of appropriate recrystallizations.

Yellow A B, an orange powder, crystallizes from alcohol in orange-red plates, melting at 104° C. It is insoluble in water, but dissolves in alcohol. The resulting orange-yellow solution is reddened by the addition of hydrochloric acid, but is not altered by the addition of sodium hydroxide. With concentrated sulphuric acid the dye forms a bluish-violet solution, which turns red and produces an orange precipitate on dilution with water. It is soluble in carbon tetrachloride and also in vegetable oils.

TABLE 7.—*Specifications for yellow A B*

Component	Maximum tolerance (ratio to pure coal-tar dye)	Minimum tolerance	Method of analysis
	Per cent	*° C.*	*Page*
Moisture	0.2		14.
Sulphated ash	.3		18.
Heavy metals	None.		19.
Arsenic as As_2O_3	0.00014		22, 23.
Pure coal-tar dye	(1)		26.
Matter insoluble in carbon tetrachloride	.5		27.
Water extractives:			
Neutral	.25		27.
Acid	.05		27.
Melting point		[2] 99	28.

[1] Although no limit is here defined, a statement of the percentage found by analysis must be incorporated in the foundation and supplemental affidavits (*70*).
[2] Corrected.

The analytical data required in the foundation and supplemental affidavits (*70*) and the methods for making the analyses are indicated in Table 7, which also shows the purity specifications necessary for certification.

YELLOW O B

Yellow O B ($C_{17}H_{15}N_3$), an oil-soluble monazo dye, is made by coupling diazotized *o*-toluidine with *β*-naphthylamine. As in the case of its lower homolog, yellow A B, it is necessary that any uncombined intermediates and any decomposition products of the diazotized base be completely removed from the final product.

It is an orange powder, which crystallizes from alcohol in orange-red plates and melts at 126°C. It is insoluble in water, but dissolves in alcohol to produce an orange-yellow solution, the color of which is reddened by hydrochloric acid but not altered by sodium hydroxide. It dissolves in concentrated sulphuric acid to form a reddish-violet solution, which turns red and produces an orange-brown precipitate when diluted with water. It is soluble in carbon tetrachloride and also in vegetable oils.

Table 8 lists the analytical determinations which must be reported in the foundation and supplemental affidavits (*70*), together with the methods to be used and the impurities tolerated in batches acceptable for certification.

TABLE 8.—*Specifications for yellow O B*

Component	Maximum tolerance (ratio to pure coal-tar dye)	Minimum tolerance	Method of analysis
	Per cent	°C.	*Page*
Moisture	0.2		14.
Sulphated ash	.3		18.
Heavy metals	None.		19.
Arsenic as As_2O_3	0.00014		22, 23.
Pure coal-tar dye	(1)		26.
Matter insoluble in carbon tetrachloride	.5		27.
Water extractives:			
Neutral	.25		27.
Acid	.05		27.
Melting point		[2] 120	28.

[1] Although no limit is here defined, a statement of the percentage found by analysis must be incorporated in the foundation and supplemental affidavits (*70*).
[2] Corrected.

GUINEA GREEN B

Guinea green B ($C_{37}H_{35}N_2O_6S_2Na$), a triphenylmethane dye (*72*), is made by condensing benzylethylanilinesulphonic acid with benzaldehyde, followed by oxidation of the resulting compound and conversion into the monosodium salt. It is necessary in manufacturing this dye to use very pure intermediates, especially benzylethylanilinesulphonic acid, which should be as free as possible from homologs. This dye is subject to contamination by organic matter, owing to side reactions, which may produce benzoic acid and condensation products other than guinea green B. For this reason its manufacture requires very careful supervision.

Guinea green B is a dull dark-green powder or bright crystalline solid. It dissolves in water to form a green solution, which turns brownish-yellow on the addition of hydrochloric acid and blackish-

green on the addition of sodium hydroxide. Owing probably to the formation of the disodium salt, the aqueous solution is decolorized by an excess of sodium hydroxide. It is decolorized by a hot solution of alkalis or alkali carbonate. It is soluble in ethyl alcohol but less soluble in amyl alcohol. With concentrated sulphuric acid it forms a yellow solution, which when diluted with water turns first yellowish-red and then green. This dye has some properties which tend to cause it to separate from solution, particularly when a sweetened solution is carbonated, in the form of a green scum or foam.

In 1889 the following patents for the preparation of guinea green B were issued: German patent 50,782 (*1*); British patent 7,550 (*1*); French patent 198,415 (*1*).

The analytical data listed in Table 9 should be included in the foundation and supplemental affidavits (*70*). The methods of analysis and the purity specifications to which the dye must conform for certification are also indicated in the table.

TABLE 9.—*Specifications for guinea green B*

Component	Maximum tolerance (ratio to pure coal-tar dye)	Minimum tolerance	Method of analysis
	Per cent	*Per cent*	*Page*
Moisture	10.0		14.
Insoluble matter:			
Total	.4		15.
Nonvolatile	.2		15.
Sodium chloride	4.0		15, 16.
Sodium sulphate	1.0		18.
Sulphated ash	(1)		18.
Sodium in color	Theoretical.		18.
Heavy metals	None.		19.
Calcium	0.3		19.
Magnesium	.3		19.
Aluminium	.1		19.
Iron	.3		19.
Arsenic as As_2O_3	.00014		21, 23.
Ether extractives:			
Neutral	(1)		23.
Alkaline	(1)		23.
Acid	(1)		24.
Total	.4		24.
Sulphur in color	Theoretical.		24.
Color acid		79.4	25.
Pure coal-tar dye		82.0	26.

[1] Although no limit is here defined, a statement of the percentage found by analysis must be incorporated in the foundation and supplemental affidavits (*70*).

LIGHT GREEN S F YELLOWISH

Light green S F yellowish ($C_{37}H_{34}N_2O_9S_3Na_2$), a triphenylmethane dye, differs from guinea green B in composition in that it contains one more sulphonic group (*16*). It is made by the condensation of benzylethylaniline with benzaldehyde, sulphonation of the product to the trisulphonic acid, oxidation of this, and conversion of the resulting compound into the mono- or disodium salt (*59*). As in the case of guinea green B (p. 10), precautions are necessary with respect to purity of the intermediates, especially benzylethylaniline. The manufacture of this dye is more difficult than that of the other permitted dyes, owing to the production, by side reactions, of other organic substances, from which it is practically impossible to purify the finished dye. In sulphonating, special care must be exercised to intro-

duce three sulphonic acid groups into the molecule without burning or charring the mass. Undersulphonation results in the production of guinea green B; oversulphonation results in the breaking down of the molecule.

Light green S F yellowish is a reddish-brown powder. It dissolves in water to form a green solution, which, upon the addition of hydrochloric acid, turns yellowish-brown and then gradually fades. Upon the addition of sodium hydroxide the aqueous solution is almost completely decolorized, yielding a dull violet precipitate. It is decolorized by a hot solution of alkalis and alkali carbonates. With concentrated sulphuric acid it forms a yellow solution, which, when diluted with water, turns green.

The methods of examination and the maximum percentages of impurities tolerated are listed in Table 10, which also shows the analytical data to be incorporated in the foundation and supplemental affidavits (*70*).

TABLE 10.—*Specifications for light green S F yellowish*

Component	Maximum tolerance (ratio to pure coal-tar dye)	Minimum tolerance	Method of analysis
	Per cent	*Per cent*	*Page*
Moisture	10.0		14.
Insoluble matter:			
Total	.2		15.
Nonvolatile	.2		15.
Sodium chloride	3.0		15, 16.
Sodium sulphate	3.0		17.
Sulphated ash	(1)		18.
Sodium in color	Theoretical.		18.
Heavy metals	None.		19.
Calcium	0.3		19.
Magnesium	.3		19.
Aluminium	.1		19.
Iron	.3		19.
Arsenic as As_2O_3	.00014		21, 25.
Ether extractives:			
Neutral	(1)		23.
Alkaline	(1)		23.
Acid	(1)		24.
Total	.4		24.
Sulphur in color	Theoretical.		24.
Color acid		77.5	25.
Pure coal-tar dye		82.0	26.
Subsidiary dyes (to be calculated as guinea green B, unless the identity of the extraneous dye or dyes is definitely known, in which case the known dye or dyes must be reported as individuals).	5.0		29.

[1] Although no limit is here defined, a statement of the percentage found by analysis must be incorporated in the foundation and supplemental affidavits (*70*).

INDIGOTINE

Indigotine ($C_{16}H_8N_2O_8S_2Na_2$), or sodium indigo disulphonate, the sodium salt of the disulphonic acid of indigo (*14*, *71*), is prepared by sulphonating indigo (*26*, *27*, *35*). Because of its more homogeneous composition, it is better to use synthetic indigo rather than the natural product for the manufacture of indigotine. Indigo of high purity should be used, and care should be taken that the sulphonation proceeds to the production of the disulphonic acid only.

Indigotine, a blue-brown or red-brown powder, dissolves in water to form a blue solution. On adding hydrochloric acid, the aqueous solution becomes bluish-violet; further dilution with water restores

the blue color, however. On adding sodium hydroxide the aqueous solution turns green to yellowish green. Indigotine is sparingly soluble in alcohol. With concentrated sulphuric acid it forms a bluish-violet solution, which turns blue when diluted with water. The color of the aqueous solution of this dye is fugitive to light.

In 1890 the following patents for the preparation of indigotine were issued: German patent 63,218 (*9*); British patent 8,726 (*7*); French patent 206,567 (*7*). In 1893 German patent 68,372 (*7*) was issued. In 1894 United States patent 524,256 (*7*) was issued.

The percentages of impurities tolerated in this dye, the analytical data needed in foundation and supplemental affidavits (*70*), and the methods for determining these data are shown in Table 11.

TABLE 11.—*Specifications for indigotine*

Component	Maximum tolerance (ratio to pure coal-tar dye)	Minimum tolerance	Method of analysis
	Per cent	*Per cent*	*Page*
Moisture	12.0		14.
Insoluble matter:			
Total	.5		15.
Nonvolatile	.4		15.
Sodium chloride	5.0		15, 16.
Sodium sulphate	2.0		17.
Sulphated ash	(1)		18.
Sodium in color	Theoretical.		18.
Heavy metals	None.		19.
Calcium	.3		19.
Magnesium	.3		19.
Aluminium	.1		19.
Iron	.3		19.
Arsenic as As_2O_3	.00014		22, 23.
Ether extractives:			
Neutral	(1)		23.
Alkaline	(1)		23.
Acid	(1)		24.
Total	.3		24.
Sulphur in color	Theoretical.		24.
Color acid		74.3	25.
Pure coal-tar dye		82.0	26.
Lower sulphonated dyes	5.0		29.

[1] Although no limit is here defined, a statement of the percentage found by analysis must be incorporated in the foundation and supplemental affidavits (*70*).

METHODS OF ANALYSIS OF PERMITTED COAL-TAR FOOD DYES

The methods of analysis of coal-tar dyes intended for use in coloring foods are subject to revision as the result of the development of more refined or simpler procedures. The methods here described are the ones now used in the Bureau of Chemistry (*13, 43, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 63*). Acknowledgment is made and thanks are extended to the manufacturers and their staffs for suggestions and criticisms in this work.

REAGENTS

In these methods the term "concentrated sulphuric acid" denotes an acid having a specific gravity of 1.84; "strong hydrochloric acid," an acid having a specific gravity of 1.184; "strong nitric acid," an acid having a specific gravity of 1.42; and "strong ammonium hydroxide," a base having a specific gravity of 0.90. In the

expressions (1 + 1), (1 + 9), etc., accompanying the names of reagents, the first numeral indicates the volume of strong or concentrated reagent used and the second numeral indicates the volume of water used.

PREPARATION OF SAMPLE

Take a 500-gram true representative sample of the batch, or one that after all tests have been made will leave enough for future reference, should any question arise. For determinations requiring a dry-weight starting point, weigh the various portions in uninterrupted succession, using wide weighing bottles for these and other weighings. Weigh out catch quantities, approximating those needed, keeping the bottles stoppered as much as possible.

Without delay prepare a master solution of such concentration and volume as will provide for all determinations, including check analyses, in which a solution of the dye as a starting point is specified. The concentration of the solution should be at least as great as the maximum required in the procedures to be followed. Aliquots, suitably diluted, are used in the procedures requiring lower concentrations.

MOISTURE

For ponceau 3R, amaranth, erythrosine, orange I, naphthol yellow S, tartrazine, guinea green B, light green S F yellowish, and indigotine.—Weigh about 2 grams of sample in a weighing bottle, 2 inches in diameter, or in an aluminum dish, 2 inches in diameter, and dry to constant weight by heating at 135° C. for six hours, or overnight. Before each weighing cool over sulphuric acid in a desiccator. From the decrease in weight calculate the percentage of moisture.

For yellow A B and yellow O B.—Proceed as directed in the foregoing paragraph, heating the dye to 80° C. instead of to 135° C. Yellow A B and yellow O B are so slightly hygroscopic that they may be weighed in the container on an even-weight basis.

TOTAL WATER-INSOLUBLE MATTER

Apparatus

Wash with acid and later with water high-grade retentive asbestos and repeatedly elutriate it to eliminate the fine particles.

Pour into a Gooch crucible placed in a filter flask enough asbestos suspension to make a mat one-eighth inch thick when packed. Connect the flask with a slow suction and pack the mat down evenly with a tamper. Remove the Gooch crucible from the filter flask and loosen the mat around the edges with a thin, narrow blade or pin; take the mat out and put it back in an inverted position, packing it down tightly as before. With this as a base, prepare a well-packed mat one-half inch thick.

If it seems probable that the percentage of total insoluble matter present is lower than the maximum permitted for nonvolatile insoluble matter, treat the crucible as follows: Wash with hot water, dry in an air oven at 135° C., cool in a desiccator containing calcium chloride, and weigh. Repeat until the weight is constant.

If it seems probable that the percentage of total insoluble matter present is higher than the maximum permitted for nonvolatile in-

soluble matter, treat the crucible as follows: Wash with hot water, ignite, rewash, dry in an air oven at 135° C., cool in a desiccator containing calcium chloride, and weigh. Repeat until the weight is constant

Determination

For amaranth, erythrosine, naphthol yellow S, tartrazine, guinea green B, and light green S F yellowish.—Dissolve 5 grams of dye in 200 c. c. of hot water and allow the solution to cool to room temperature. Filter through the prepared Gooch crucible and wash with cold water until all the dissolved dye has been removed. Dry in an air oven at 135° C., cool in a desiccator containing calcium chloride, and weigh. Repeat the drying, cooling, and weighing until the weight is constant.

For ponceau 3R, orange I, and indigotine.—Dissolve 5 grams of dye in 250 c. c. of hot water and boil, with frequent stirring, for three minutes. Cool the solution to room temperature and let stand cold overnight. Filter with moderate suction. Wash with cold water, dry in an air oven at 135° C., cool in a desiccator containing calcium chloride, and weigh. Repeat the drying, cooling, and weighing until the weight is constant.

In both cases report the percentage increase in weight as total water-insoluble matter.

NONVOLATILE WATER-INSOLUBLE MATTER

For ponceau 3R, amaranth, erythrosine, orange I, naphthol yellow S, tartrazine, guinea green B, light green S F yellowish, and indigotine.—Incinerate the Gooch crucible containing the total insoluble matter (p. 14) at a low red heat until all the organic matter has been volatilized. Cool in a desiccator containing calcium chloride and weigh. Report as nonvolatile water-insoluble matter.

SODIUM CHLORIDE

Reagents

All reagents must be halogen free.

Sulphur dioxide solution.—Saturate ice-cold distilled water with sulphur dioxide. Keep the solution stoppered and in a cold place.

Determination

For ponceau 3R, amaranth, orange I, tartrazine, guinea green B, light green S F yellowish, and indigotine.—Thoroughly mix 5 grams of dye with 4 to 6 grams of potassium or sodium carbonate in a 50 c. c. platinum or nickel crucible and moisten with water or 50 per cent alcohol. Cover evenly with about 1 gram of the powdered carbonate, dry, and ignite at a low red heat until all organic matter is destroyed. Allow to cool and add enough water to form a thin paste. With a glass rod break up any lumps present, in order to produce a uniform suspension. Wash the mixture into a 250 c. c. volumetric flask with 100 to 150 c. c. of hot water and let stand until all soluble salts have dissolved and the mixture is cold. Dilute to the mark with water, mix thoroughly, and filter through a dry

paper. Place a 200 c. c. portion of the filtrate in a 600 c. c. beaker and add enough of a 6 to 7 per cent solution of potassium permanganate to oxidize the sulphides and produce a faint but permanent pink color. Add about 50 c. c. of water and a slight excess of 10 per cent silver nitrate solution. (Usually 6 to 8 c. c. is enough to precipitate the chloride.) Partially cover the beaker with a watch glass and acidify the solution by carefully adding about 12 c. c. of strong nitric acid. Heat nearly to boiling and then add the saturated sulphur dioxide solution slowly and with stirring until the oxides of manganese have dissolved, leaving the silver chloride white. Boil until the excess of sulphur dioxide is removed, cool, and filter through a weighed Gooch crucible. Wash the silver chloride with hot water, which is slightly acidified with nitric acid, dry the crucible and its contents at 105° to 110° C., cool in a desiccator, and weigh. Calculate to percentage of sodium chloride.

For erythrosine.—Dissolve 5 grams of the dye in 400 c. c. of water and transfer the solution to a 500 c. c. volumetric flask. Precipitate the color acid by adding a mixture of 2 c. c. of strong nitric acid and 10 to 20 c. c. of water. Dilute to 500 c. c., mix, and filter through a dry paper. Reserve 100 c. c. of the filtrate for possible use in determining sodium sulphate in erythrosine (p. 17). Treat 200 c. c. of the filtrate with slightly more silver nitrate solution than is required to precipitate the halogens present. Add 5 c. c. of strong nitric acid and heat to boiling. After cooling, collect the precipitate in a weighed Gooch crucible. Wash, dry, and weigh as directed in the first paragraph of this section. If a test shows that sodium iodide is present, determine its weight as directed on page 30 and subtract the weight of silver iodide from the weight of the precipitate. Calculate the percentage of sodium chloride from the net silver chloride.

For naphthol yellow S.—Dissolve 5 grams of the dye in 250 c. c. of water and filter if not clear. Add 5 c. c. of strong nitric acid and precipitate the chloride by adding a slight excess of 10 per cent silver nitrate solution. Boil for a few minutes, cool, and filter through a weighed Gooch crucible. Wash, dry, and weigh the precipitate and calculate as directed in the first paragraph of this section.

For ponceau 3R.—Dissolve 5 grams of the dye in 150 c. c. of hot water, wash into a 250 c. c. volumetric flask, and add 25 c. c. of a 10 per cent solution of barium nitrate. Cool the mixture, make up to the mark, mix, and filter through a dry paper. Acidify 100 c. c. of the filtrate (representing 2 grams of dye) with strong nitric acid and treat with a slight excess of 10 per cent silver nitrate solution. Collect the silver chloride on a weighed Gooch crucible. Wash, cool in a desiccator, weigh, and calculate as directed in the first paragraph of this section.

For ponceau 3R, amaranth, orange I, naphthol yellow S, tartrazine, guinea green B, light green S F yellowish, and indigotine.—In a nickel crucible mix 1 gram of the dye with about 15 grams of pure sodium peroxide. Place the cover on the crucible and start the fusion by introducing a red-hot iron wire through a hole previously bored in the cover. When the reaction is complete allow the crucible to cool. (If the fusion is too rapid, repeat the entire procedure, using a larger proportion of sodium peroxide.) Wash the cover over a 600 c. c. beaker with 50 to 75 c. c. of water. Place the crucible on its side in the beaker and add water until the crucible is about half covered.

When the mixture is dissolved remove and wash the crucible over the beaker. Neutralize the solution carefully with strong nitric acid, adding 1 to 3 c. c. in excess. Cool, transfer to a 6-inch porcelain evaporating dish, and add 19 c. c. of 0.1 N silver nitrate solution and 5 to 10 c. c. of 10 per cent ferric alum solution. Titrate the solution with 0.1 N ammonium or potassium thiocyanate, adding 1 or 2 drops after the red end point has been reached. Add another cubic centimeter of silver nitrate, making 20 c. c. in all. Filter the solution through a Büchner funnel and wash the precipitate with a little water slightly acidified with nitric acid. Return the filtrate and washings to the evaporating dish and titrate to the end point with ammonium or potassium thiocyanate. Calculate to percentage of sodium chloride.

SODIUM SULPHATE

For ponceau 3R, amaranth, orange I, tartrazine, and indigotine.—Transfer to a 250 c. c. volumetric flask that volume of the master solution which contains 5 grams of the dye. Add water, if necessary to bring the volume to 200 c. c., and heat on a steam bath. Add pulverized sodium chloride free from sulphates as follows: For amaranth and tartrazine, 70 grams; for ponceau 3R, orange I, and indigotine, 50 grams. Stopper the flask and shake at frequent intervals for an hour. To hasten the precipitation the solution may be cooled in ice water. Dilute to the mark with saturated sodium chloride solution, shake, and filter on a dry 18 cm. paper. To 100 c. c. of the filtrate add 200 c. c. of water and 1 c. c. of hydrochloric acid (1+9). Heat to boiling and add a slight excess of hot 10 per cent barium chloride solution. Allow to stand overnight and filter through a weighed Gooch crucible. Wash the precipitate of barium sulphate thoroughly with hot water, ignite, cool in a desiccator containing calcium chloride, and weigh. Calculate the percentage of sodium sulphate equivalent to the barium sulphate obtained.

For erythrosine.—Transfer to a 500 c. c. tall beaker that volume of the master solution which contains 1 gram of the dye. If the quantity taken is less than 100 c. c. add water to bring the solution to that volume. Add 50 c. c. of dilute nitric acid (1+49) to precipitate the color acid, stir, and let settle. Filter off the dye and wash once, collecting the washings with the filtrate. Over another 500 c. c. beaker dissolve the dye by passing through the filter paper as little dilute ammonium hydroxide solution (1+1) as is necessary and add water to bring the volume to 100 c. c. Neutralize the base and add nitric acid (1+49) to reprecipitate the color acid. Filter off the dye and wash once, collecting the filtrate and washings with those obtained after the first precipitation of color acid. (A 100 c. c. aliquot free of color acid may be substituted, as directed in the first paragraph of this section.) Precipitate and determine the barium sulphate as directed in the first paragraph of this section.

For light green S F yellowish.—Transfer to a 500-c. c. tall beaker that portion of the master solution which contains 2 grams of the dye. Add water, if necessary, to bring to a volume of 200 c. c. Heat to about 70° C. and filter. Wash the residue and paper with hot water. Dilute the combined filtrate and washings to about 250 c. c., and add 5 c. c. of dilute hydrochloric acid (1+9). Complete

the determination and calculate as directed in the first paragraph of this section, beginning with "Heat to boiling and add a slight excess of hot 10 per cent barium chloride solution."

For naphthol yellow S.—Transfer to a 500 c. c. volumetric flask that volume of the master solution which contains 5 grams of dye. Add water, if necessary, to bring to a volume of about 300 c. c. Add hot saturated potassium chloride solution to bring to the 500-c. c. mark. Shake frequently until practically all the dye is precipitated. After the mixture is cold dilute with water to 500 c. c. Shake and filter through a dry paper. Using 100 c. c. of the filtrate, precipitate, determine, and calculate as directed in the first paragraph of this section, beginning with "To 100 c. c. of the filtrate add 200 c. c. of water and 1 c. c. of hydrochloric acid (1+9)."

For guinea green B.—Transfer to a 500 c. c. volumetric flask that volume of the master solution which contains 5 grams of the dye. Add water, if necessary, to bring the volume to 400 c. c. Add 8 c. c. of strong ammonium hydroxide and 125 grams of sodium chloride (free from sulphates) and dilute to 500 c. c. with a saturated solution of sodium chloride. Shake vigorously to precipitate the dye and filter through a dry paper. Neutralize 200 c. c. of the filtrate with dilute hydrochloric acid (1+9) and add 5 c. c. in excess. Complete the determination and calculate as directed in the first paragraph of this section, beginning with "Heat to boiling and add a slight excess of hot 10 per cent barium chloride solution."

SULPHATED ASH

For ponceau 3R, amaranth, erythrosine, orange I, naphthol yellow S, tartrazine, guinea green B, light green S F yellowish, and indigotine.—Weigh accurately about 5 grams of dye in a weighing bottle. Transfer to a pyrex Kjeldahl flask, washing out the weighing bottle with a little water. Destroy the organic matter to a convenient extent by nitric and sulphuric acid digestion, using 15 c. c. of concentrated sulphuric acid and adding concentrated nitric acid as required. As most of the nitric acid is driven off lower the flame to avoid local overheating. Transfer the mixture to a weighed platinum dish and heat over a ring burner, using at first a low flame at a safe distance below the dish, increasing the flame and bringing it closer to the dish by gradual steps. Thus continue the destruction of the organic matter and the volatilization of the acids. Continue the heating until the production of acid fumes decreases and danger of loss of volatile metallic elements arises. If carbon remains remove the flame, let the mass cool a little, and add concentrated sulphuric acid, drop by drop, until the mass is moistened. Repeat the treatment until the carbon is burned off and the ash is white or reddish. Heat carefully with a blast lamp until fusion takes place with the production of a clear liquid free from bubbles. Cool in a desiccator and weigh. After deducting the weight of sodium sulphate equivalent to the inorganic sodium salts (chlorides, sulphates, carbonates, etc.) found in the other determinations, calculate to percentage of sodium combined in the dye. Reserve the sulphates for the determination of heavy metals.

For yellow A B and yellow O B.—In a weighed platinum dish place 5 grams of dye. (Yellow A B and yellow O B are so slightly

hygroscopic that they may be weighed in the container on an even-weight basis.) Heat at a low temperature until most of the dye has been volatilized. Moisten the residue with concentrated sulphuric acid and complete the procedure as directed in the first paragraph of this section, beginning with "and heat over a ring burner." Inasmuch as yellow A B and yellow O B contain no metallic element, the presence of any sulphated ash is due to impurities. This sulphated ash, although not subject to calculation in terms of sodium or other base of the color, is reserved for the determination of heavy metals.

HEAVY METALS

For ponceau 3R, amaranth, erythrosine, orange I, naphthol yellow S, tartrazine, guinea green B, light green S F yellowish, and indigotine.—Moisten with 5 c. c. of strong hydrochloric acid the sulphated ash obtained in the sulphated-ash determination and evaporate to dryness on a steam bath. Warm the residue with 20 c. c. of hydrochloric acid (1+19) until all soluble material is dissolved. Transfer to a 100 c. c. volumetric flask, dilute to the mark, mix, and filter through a dry paper. Reserve two 40 c. c. portions for the iron, aluminium, calcium, and magnesium determinations. Pour the rest of the filtrate into a test tube and pass in a washed stream of hydrogen sulphide for 30 minutes. No turbidity other than that due to precipitated sulphur should appear. If a colored precipitate is formed, filter and test it for copper and tin. Make the solution slightly alkaline with ammonium hydroxide (1+1). A white precipitate indicates the presence of zinc, which is not tolerated; a marked coloration indicates that the quantity of iron should be determined.

For yellow A B and yellow O B.—The color of the sulphated ash, obtained as directed above, shows whether the ash is mainly iron oxide or silica. In either case fuse it with 1 gram of potassium carbonate (reagent quality) until the silicates have been decomposed. Moisten the residue with 2 or 3 c. c. of strong hydrochloric acid, evaporate to dryness on a steam bath, and treat as directed in the first paragraph of this section. If only a trace of iron oxide or silica is present in the original ash, the fusion with potassium carbonate may be omitted.

IRON, ALUMINIUM, CALCIUM, AND MAGNESIUM

For ponceau 3R, amaranth, erythrosine, orange I, naphthol yellow S, tartrazine, yellow A B, yellow O B, guinea green B, light green S F yellowish, and indigotine.—To one of the two portions reserved as directed above add 5 grams of ammonium chloride and neutralize with ammonium hydroxide (1+1), boiling to drive off any excess. If the precipitate is very slight, it may be neglected; otherwise, filter through a quantitative paper, wash (reserving the filtrates and washings), and ignite paper and precipitate in a weighed crucible. Weigh the mixture of iron oxide (Fe_2O_3) and aluminium oxide (Al_2O_3). Place the mixed oxides in a 500 c. c. Erlenmeyer flask and dissolve in aqua regia, boiling to drive off chlorine. Add water to bring the volume to about 75 c. c. and add ammonium hydroxide to incipient precipitation. Dissolve the precipitate with as little hydrochloric acid as possible and cool. Titrate the ferric iron present with 0.1

N titanium trichloride solution (p. 24), using 5 grams of ammonium thiocyanate as indicator. Calculate the iron as ferric oxide (Fe_2O_3). To calculate the quantity of aluminium oxide (Al_2O_3) deduct the weight of ferric oxide from the total weight of mixed oxides. Calculate the percentage of iron and aluminium from the weights of the oxides.

To the other reserved portion add 250 c. c. of water to insure a low concentration of magnesium, if present. Heat to boiling and add 3.5 grams of ammonium chloride and 1 gram of oxalic acid. Add ammonium hydroxide solution (1+99) until the solution is just neutral. Cool and let stand for an hour. Then filter through an asbestos mat prepared on a small Witt plate in a glass funnel and wash with very little water, reserving the combined liquids. Place the mat in a beaker and add 100 c. c. of water and 2 c. c. of concentrated sulphuric acid. Heat gently until the calcium oxalate dissolves. Titrate with 0.1 N potassium permanganate solution. Calculate as percentage of calcium.

Heat to boiling the reserved filtrate and washings and add N sodium ammonium hydrogen phosphate solution until there is no further precipitation. While stirring add about one-third the volume of ammonium hydroxide solution (1+9). Let stand three hours, filter through an ashless paper, and wash with ammonium hydroxide solution (1+49). Convert to magnesium pyrophosphate by the usual method. Cool in a desiccator and weigh the magnesium pyrophosphate. Calculate as percentage of magnesium.

ARSENIC

Reagents

Strong nitric acid.—Arsenic-free and having a specific gravity of 1.42.

Concentrated sulphuric acid.—Arsenic-free and having a specific gravity of 1.84.

Ammonium hydroxide.—Arsenic-free and having a specific gravity of 0.90.

Dilute hydrochloric acid.—Arsenic-free and containing 10 per cent by weight of hydrogen chloride.

Dilute nitric acid.—Arsenic-free (1+9).

Sodium phosphate solution.—Arsenic-free and containing 100 grams of the crystallized salt per liter or an equivalent quantity of phosphoric acid.

Magnesia mixture.—Arsenic-free and containing per liter 55 grams of hydrated magnesium chloride, 55 grams of ammonium chloride, and 88 c. c. of strong ammonium hydroxide.

Saturated bromine water.

Zinc.—Break arsenic-free stick zinc 6 mm. wide into pieces approximately 1 cm. long.

Lead acetate paper.—Soak heavy filter paper in 20 per cent lead acetate solution, dry, and cut into pieces about 4.5 by 16 cm.

Lead acetate cotton.—Soak absorbent cotton in 5 per cent lead acetate solution.

Mercuric bromide paper.—Cut heavy, close-textured drafting paper into strips exactly 2.5 mm. wide and about 12 cm. long. Soak

for an hour in a 5 per cent solution of mercuric bromide in 95 per cent alcohol, squeeze out the excess solution, and dry on glass rods. (In the preparation care must be taken to protect the paper from contact with the table.) The ends of the strips should be cut off before using.

Potassium iodide solution.—Dissolve 20 grams of potassium iodide in distilled water and dilute to 100 c. c.

Stannous chloride solution.—Dissolve 40 grams of arsenic-free stannous chloride crystals in enough strong hydrochloric acid to make 100 c. c.

Standard arsenic solution.—Dissolve 1 gram of arsenious oxide in 25 c. c. of 20 per cent sodium hydroxide solution, neutralize with dilute sulphuric acid, add, in excess, 10 c. c. of concentrated sulphuric acid, and dilute to 1 liter with air-free water. One cubic centimeter of this solution contains 1 mg. of arsenious oxide (As_2O_3). Dilute 20 c. c. of this solution to 1 liter. Diluting 50 c. c. of this second solution to 1 liter gives a final solution which contains 0.001 mg. of arsenious oxide (As_2O_3) per cubic centimeter. This is used to prepare the standard stains. The dilute solutions must be prepared immediately before use.

Apparatus

Connect the generator (fig. 1), a 2-ounce wide-mouth bottle, by a perforated rubber stopper to a glass tube, 1 cm. in diameter and 6 cm. long, containing a piece of the lead acetate paper rolled into a spiral. Connect this tube by a perforated rubber stopper with a similar tube filled with the lead acetate cotton, squeezed to remove excess of the solution. The cotton in all determinations must be uniformly moist in order to obtain comparable stains. Connect the second tube by a perforated rubber stopper to a narrow glass tube, 3 mm. in internal diameter and 12 cm. long, containing a strip of the mercuric bromide paper. Rubber stoppers used for connections must be free from white coating.

Determination by Direct Precipitation

For amaranth, naphthol yellow S, tartrazine, guinea green B, and light green S F yellowish.—Dissolve 10 grams of the dye in 250 c. c. of water and add 10 c. c. of the bromine water. Make the mixture alkaline with 1 to 2 c. c. of the strong ammonium hydroxide. Then add 20 c. c. of the sodium phosphate solution and a slight excess of the magnesia mixture. The quantity of magnesia mixture used must be from 1 to 5 c. c. in excess of that required to completely precipitate the phosphate, as ascertained previously by experiment. Add the magnesia mixture slowly, stirring the solution well during the addition. Add 10 c. c. of the ammonium hydroxide and allow the mixture to stand for at least 30 minutes. Filter through an 18 cm. paper and wash with dilute ammonium hydroxide (1 + 9) until practically all dye is removed. Then wash with about 5 c. c. of water. Allow the filter containing the washed precipitate to drain for 15 to 30 minutes to remove most of the adhering liquid. Finally dissolve the magnesium ammonium phosphate and arsenate by pouring 40 c. c. of 10 per cent hydrochloric acid over the filter in small portions and letting it drain into the generator bottle. Add 4 c. c. of

the 20 per cent potassium iodide solution. Heat to about 90° C., add 3 drops of the stannous chloride solution, and keep the solution at 90° C. for 10 minutes. Cool the generator and its contents, first in air and then to about 5° C. in a pan containing water and ice. Add about 15 grams of the stick zinc and quickly connect the entire apparatus. Keep the generator in ice water for 15 minutes. Remove from the bath and let the evolution of gas continue for an hour longer. Finally, place the generator bottle on a hot plate and heat cautiously for about 30 minutes. Remove the sensitized paper and compare the stain with similar stains produced under like conditions with known quantities of arsenic, using portions of the standard arsenic solution containing 0.001, 0.002, 0.005, 0.010, 0.015, 0.025, and 0.030 mg. of arsenious oxide (As_2O_3). Conduct a blank test on the reagents alone. The blank should not exceed 0.001 mg. Calculate as parts per million of arsenious oxide.

FIG. 1.—Apparatus for determination of arsenic

For erythrosine.—Dissolve 18 grams of the dye in 425 c. c. of water and add 5 c. c. of the bromine water and 20 c. c. of dilute hydrochloric acid (1+9). Mix, filter, and treat 250 c. c. of the filtrate (corresponding to 10 grams of dye) with the ammonium hydroxide, using enough to make it slightly alkaline (usually about 5 c. c.). Complete the determination and calculation as directed in the first paragraph of this section, beginning with "Then add 20 c. c. of the sodium phosphate solution."

Determination After Treatment With Nitric Acid

For ponceau 3R, amaranth, erythrosine, orange I, naphthol yellow S, tartrazine, and indigotine.—Place 12.5 grams of the powdered dye in a 600 c. c. pyrex beaker, add 25 c. c. of the strong nitric acid, and, if the dye tends to clot or cake, stir the mixture thoroughly. Boil for about five minutes, add 150 to 200 c. c. of water, and dilute the mixture to 250 c. c. in a graduated cylinder. Pour the mixture back into the beaker. Allow it to stand for a few minutes and then filter through an 18 cm. paper. Treat 200 c. c. of the filtrate (corresponding to 10 grams of dye) with the strong ammonium hydroxide, using a measured quantity sufficient to make the solution slightly alkaline (about 25 c. c.). Complete the determination and calculation as directed in the first paragraph of this section, beginning with "Then add 20 c. c. of the sodium phosphate solution."

For yellow A B and yellow O B.—In a 400 c. c. beaker mix thoroughly 12.5 grams of the powdered dye with 200 c. c. of nitric acid (1+9) and heat to boiling for 5 to 10 minutes. Allow to cool, add 25 c. c. of the strong ammonium hydroxide, dilute to 250 c. c. in a graduated cylinder, and filter. Determine the arsenic in a 200 c. c. portion of the filtrate (corresponding to 10 grams of dye) and calculate as directed in the first paragraph of this section, beginning with "Then add 20 c. c. of the sodium phosphate solution."

Determination as Total Arsenic

For ponceau 3R, amaranth, erythrosine, orange I, naphthol yellow S, tartrazine, yellow A B, yellow O B, guinea green B, light green S F yellowish, and indigotine.—Weigh 10 grams of the dye into a 600 c. c. Kjeldahl flask or tall 600 c. c. beaker provided with a cover. Treat with 15 c. c. of the concentrated sulphuric acid and 25 c. c. of the strong nitric acid and digest slowly under a hood until the nitric acid has been decomposed or volatilized and the mixture turns very dark. Cautiously add a few cubic centimeters of the strong nitric acid to the hot mixture, which will again become light yellow or orange, and heat to charring. Repeat the nitric acid treatment and the heating until the solution no longer shows a tendency to darken and remains yellow or colorless when evaporated until sulphur trioxide fumes are evolved. Allow the mixture to cool, add 200 c. c. of water, and make slightly alkaline with the strong ammonium hydroxide. Determine the arsenic in the solution and calculate as directed in the first paragraph of this section, beginning with "Then add 20 c. c. of the sodium phosphate solution." The comparatively large quantities of reagents necessary for the destruction of the organic matter will usually contain appreciable quantities of arsenic, for which correction must be made by determinations on blanks.

ETHER EXTRACTIVES

Reagents

Washed ether.—Wash ether successively with three portions of water. For 1 liter of ether use three 150 c. c. portions of water.

Dilute sodium hydroxide solution.—1 c. c. of 0.1 N alkali to 100 c. c. of water.

Dilute hydrochloric acid.—1 c. c. of 0.1 N acid to 100 c. c. of water.

Determination

For ponceau 3R, amaranth, orange I, naphthol yellow S, tartrazine, guinea green B, light green S F yellowish, and indigotine.—To determine the neutral extractives, proceed as follows: Place in a separatory funnel that volume of the master solution which contains 10 grams of the dye and add water, if necessary, to bring the volume to 150 c. c. Extract with two successive 100 c. c. portions of the washed ether, shaking for one minute during each extraction. Remove the ether by decantation into a clean funnel and rinse the first funnel with 5 c. c. of ether, decanting into the second funnel. Reserve the color solution. Wash the combined extracts successively with 35, 20, and 10 c. c. of water. Drain the ether into a beaker, rinse the funnel with 5 c. c. of ether, and drain into the same beaker. Place the beaker in a dust-free atmosphere, allow the ether to evaporate to a volume of 50 c. c., and transfer to a weighed flat-bottom 100 c. c. dish, previously dried to constant weight over sulphuric acid in a desiccator. Rinse the beaker with 5 c. c. of ether and drain into the same dish. Let the remainder of the ether evaporate, and dry over sulphuric acid to constant weight. Calculate as percentage of neutral ether extractives.[2]

To determine the alkaline extractives, proceed as follows: Prepare the color solution as directed in the foregoing paragraph, using graduated cylinders in place of the funnels, if the emulsions which

[2] Run two blanks on the washed ether and deduct the average result.

are usually formed may thus be more easily destroyed. To the reserved color solution add 2 c. c. of a 10 per cent sodium hydroxide solution and extract and rinse with ether. Reserve the color solution. Wash the combined ether extractives and rinsings successively with 35 and 10 c. c. of the dilute sodium hydroxide solution. Evaporate the ether, dry, and weigh. Calculate as percentage of alkaline ether extractives.[3]

To determine the acid extractives, proceed as follows: To the color solution reserved from the alkaline extraction add twice the volume of hydrochloric acid (1+3) necessary to neutralize. Repeat the procedure as directed for the neutral extractives, but do not reserve the color solution. Wash the ether extractive with the dilute hydrochloric acid. Calculate as percentage of acid ether extractives.[3]

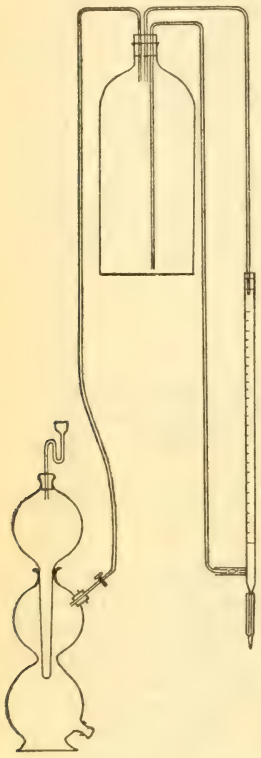

FIG. 2.—Apparatus for titration with standard titanium trichloride solution

For erythrosine.—Determine as directed in the first paragraphs of this section, omitting the acid extraction.

SULPHUR IN COLOR

For ponceau 3R, amaranth, orange I, naphthol yellow S, tartrazine, guinea green B, light green S F yellowish, and indigotine.—Determine the sulphur content by the Carius method (*17*). Deduct the sulphur equivalent to the sodium sulphate as determined on p. 17. Calculate as percentage of sulphur in color.

For ponceau 3R, amaranth, orange I, naphthol yellow S, tartrazine, guinea green B, light green S F yellowish, and indigotine.—Place about 0.2 gram of the sample in a Parr calorimetric bomb and mix thoroughly with approximately 10 grams of sodium peroxide. Add a few milligrams of sulphur-free sugar, if necessary, to aid in igniting the mass. Close the bomb and ignite. When cold open the bomb, place it in a 400 c. c. beaker, and cover the beaker with a watch glass. Add water through the lip of the beaker until the bomb is covered. When the mass is completely dissolved, remove the bomb from the beaker and wash it carefully with water, uniting the washings with the solution. Cautiously acidify the solution with dilute hydrochloric acid and filter, if necessary. Determine the sulphuric acid as directed on page 17. Deduct the sulphur equivalent to the sodium sulphate (p. 17).

COLOR ACID

REAGENTS

Standard titanium trichloride solution.—Add 150 c. c. of strong hydrochloric acid to 200 c. c. of commercial 15 per cent titanium trichloride solution and dilute to 2 liters. Titrate the required

[3] Run two blanks on the washed ether and deduct the average result.

volume against 10 c. c. of 0.1 N ferric ammonium sulphate solution, using 5 grams of pure ammonium thiocyanate as an indicator. Make the solution approximately 0.1 N and place it in a container provided with a hydrogen atmosphere (fig. 2). Take special care to have all joints airtight and cover the stoppers (preferably counter sunk) with suitable wax. Let stand for two days for absorption of residual oxygen. Standardize against 20 c. c. of 0.1 N ferric ammonium sulphate, using 5 grams of ammonium thiocyanate as indicator and titrating in a 500 c. c. Erlenmeyer flask, with a protecting stream of carbon dioxide. Preserve in an atmosphere of hydrogen.

Indicator.—For many dyes the titanium trichloride titration end point is indicated by a sharp decolorization. For some dyes the change is so gradual that an excess of titanium trichloride (not more than 0.3 c. c. of approximately 0.1 N solution) is required and a suitable standard solution of some other dye must be used for the back titration, methylene blue serving well for this purpose. In other cases it is better to use an indicator which is reduced after the original dye has reacted with the titanium trichloride. Thus a known quantity of light green S F yellowish is used as an indicator for the naphthol yellow S titration.

TABLE 12.—*Quantities of color acids equivalent to 1 c. c. of standard titanium trichloride solution*

Dye	Molecular weight of color acid	Color acid equivalent to 1 c. c. 0.1 N $TiCl_3$	Dye	Molecular weight of color acid	Color acid equivalent to 1 c. c. 0.1 N $TiCl_3$
		Gram			*Gram*
Ponceau 3R	450.4	0.01126	Tartrazine	468.3	0.01171
Amaranth	538.4	.01346	Guinea green B	668.5	.03342
Orange I	328.3	.008207	Light green S F yellowish	748.7	.03743
Naphthol yellow S	314.2	.002618	Indigotine	422.3	.02112

DETERMINATION BY TITRATION WITH TITANIUM TRICHLORIDE

For amaranth.—Into a 500 c. c. Erlenmeyer flask pipet that volume of the master solution which corresponds to 20 c. c. of 0.1 N titanium trichloride solution (Table 12). Add water, if necessary, to bring the volume to 50 c. c. and acidify with 1.5 c. c. of strong hydrochloric acid. Pass in a stream of carbon dioxide and heat to vigorous boiling. Then, without delay, titrate with the standardized titanium trichloride solution, keeping the carbon dioxide flow continuous to the end. No indicator is required; the end point is shown by the disappearance of the characteristic color of the dye. Calculate as percentage of color acid.

For ponceau 3R, orange I, tartrazine, and indigotine.—Proceed as directed in the foregoing paragraph, substituting 15 grams of sodium acid tartrate for hydrochloric acid and bringing the volume to 100 c. c. First completely dissolve the tartrate by boiling; then add the dye solution and boil again under carbon dioxide. Run blanks on 15 grams of the acid tartrate, using 100 c. c. of water and, as indicator, 1 mg. of the dye concerned. Calculate as percentage of color acid.

For guinea green B and light green S F yellowish.—Dissolve 5 grams of the dye in 400 c. c. of water previously boiled and cooled under

carbon dioxide. Transfer to a 500 c. c. volumetric flask and dilute to the mark with air-free water. In a 1-liter Erlenmeyer flask dissolve 30 grams of sodium acid tartrate in 200 c. c. of water, boil to expel air, and, under carbon dioxide, cool to 85° C. Add 100 c. c. of the dye solution and titrate under carbon dioxide at 60° to 70° C. Run blanks on 30 grams of the sodium acid tartrate in 300 c. c. of water, using 1 mg. of the dye as indicator. Calculate as percentage of color acid.

For naphthol yellow S.—Proceed as directed in the second paragraph of this section, using as an indicator that volume of light green S F yellowish standardized solution (freshly made) which contains 10 mg. of dye. Run a blank on the tartrate, light green S F yellowish, and water. Calculate as percentage of color acid.

Determination by Precipitation (*28*)

For erythrosine.—Dilute to 100 c. c. that volume of the master solution which contains 0.25 gram of dye. Add 5 c. c. of nitric acid (approximately 0.6 N strength) and filter through a weighed Gooch crucible. Wash thoroughly with 0.5 per cent nitric acid and finally with not more than 10 c. c. of distilled water. The precipitate should not be allowed to cake in the crucible until the washing has been completed. Dry to constant weight at 135° C. Calculate as percentage of color acid.

PURE COAL-TAR DYE

Determination by Titration with Titanium Trichloride

For yellow A B and yellow O B.—Dissolve 15 grams of sodium acid tartrate in 100 c. c. of water and add 0.1 gram of dye dissolved in 100 c. c. of 95 per cent alcohol. Titrate with the standard titanium trichloride (p. 24) under carbon dioxide, using as an indicator 10 mg. of light green S F yellowish from a fresh standardized solution, as directed in the color acid determination for naphthol yellow S. Run a blank as directed in that determination, including also the 100 c. c of 95 per cent alcohol. One cubic centimeter of 0.1 N titanium trichloride is equivalent to 0.006180 gram of yellow A B and to 0.006530 gram of yellow O B. Calculate as percentage of dye.

Determination by Calculation from Color Acid Determination

For ponceau 3R, amaranth, orange I, naphthol yellow S, tartrazine, quinea green B, light green S F yellowish, and indigotine.—Multiply by the factor 0.956 the percentage of sodium combined in the dye as determined for sulphated ash (p. 18), and add the result to the percentage of color acid. Report as percentage of dye.

For erythrosine.—Multiply by the factor 1.074 the percentage of color acid. Report as percentage of dye.

Determination by Titration with Potassium Permanganate

For indigotine.—Dilute to 400 c. c. that volume of the master solution which contains 0.04 gram of dye. Add 2 c. c. of concentrated sulphuric acid and titrate against standard approximately 0.02 N potassium permanganate solution. The end point is shown by the production of a clear yellow color. The titer of the standard solution

must be fixed by titration against a freshly made solution of indigotine of known purity, the same conditions of concentration and acidity being maintained. Calculate as percentage of dye.

MATTER INSOLUBLE IN CARBON TETRACHLORIDE

For yellow A B and yellow O B.—In a 100 c. c. beaker mix 5 grams of the dye with 50 c. c. of carbon tetrachloride, stir, and heat to boiling. With carbon tetrachloride wash to constant weight a Gooch crucible prepared as directed on page 14, except that ignition is omitted. Filter the hot dye solution through the crucible, transferring to it the residue in the beaker, and wash with five 10 c. c. portions of carbon tetrachloride. Dry at 100° to 105° C. and weigh. Report the percentage increase in weight as matter insoluble in carbon tetrachloride.

WATER EXTRACTIVES

For yellow A B and yellow O B.—To determine the neutral extractives, proceed as follows: Place 5 grams of the well-powdered dye in a 500 c. c. Erlenmeyer flask or wide-mouth bottle, add 200 c. c. of water, and stopper. Mix by shaking vigorously several times during 2 hours. Filter the mixture, reserving the dye, and evaporate 100 c. c. of the filtrate in a weighed platinum dish on a steam bath. Dry in an oven at 100° to 105° C., cool in a desiccator, and weigh. Report the percentage increase in weight as neutral water extractives. Test small portions of the remainder of the filtrate for chlorides, sulphates, and nitrates. If more than traces are present, make the proper analyses on aliquot portions of the filtrate.

To determine the acid extractives, proceed as follows: In 100 c. c. of benzene dissolve the dye reserved from the neutral extractive determination. Extract in a separatory funnel with two successive 25 c. c. portions of hydrochloric acid (1 + 9). Combine the extracts and wash once with 25 c. c. of benzene. Draw off the acid extract into a weighed platinum dish. Discard the benzene and wash the funnel with 10 c. c. of hydrochloric acid (1 + 9), adding this washing to the extract in the dish. Evaporate to dryness on the steam bath, cool in a desiccator containing calcium chloride, and weigh. Report the percentage increase in weight as acid water extractives.

MELTING POINT

Apparatus

Set up the apparatus (fig. 3), consisting of a tube about 15 cm. long and 3.5 cm. in internal diameter, with a bulb of 5 cm. internal diameter. Fill the tube with glycerin or sulphuric acid to about the height indicated. Fit the tube with a cork stopper carrying a glass tube (*A*), 5 mm. in diameter, which reaches nearly to the bottom of the bath, an ordinary test tube (*B*), in which a standardized thermometer (*C*) is suspended by means of a rubber stopper in such a manner that the mercury column is wholly within the tube and the mercury bulb equidistant from its walls, a long-stemmed thermometer (*D*), supported so as to reach a short distance below the tube *B*, and an outlet tube (*E*) to permit the escape of air and vapor.

DETERMINATION

For yellow A B and yellow O B.—To a capillary tube of 1 mm. or smaller internal diameter, sealed at one end, transfer a small portion of the sample by inserting into the sample the open end of the capillary tube, removing, inverting, and gently tapping until the loosely packed substance fills the bottom of the tube to a height of 2 to 4 mm. Attach the capillary tube to the thermometer *C* by means of a small rubber band, so that the sample is placed at about the middle of the mercury bulb. Replace the thermometer in the tube, connect the tube *A* to the air blast, and force a not too rapid stream of air bubbles through the bath. Raise the temperature of the bath rapidly to within 5° of the approximate melting point of the sample. Keep the temperature constant until the thermometer reading is within 1° of that of the bath. Then raise the temperature slowly until melting is observed. On approaching within approximately 0.5° of the melting point the substance darkens; true melting is indicated by the formation of a meniscus on the upper surface. When this condition is observed record the temperature. Keep the temperature as nearly constant as possible until the whole of the sample has liquefied; then record the temperature again.

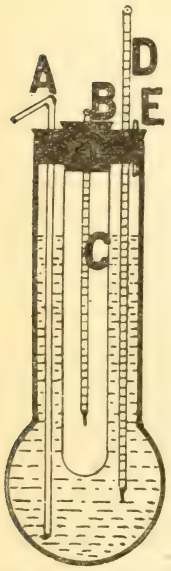

FIG. 3.—Melting point apparatus

LOWER SULPHONATED DYES

For amaranth and tartrazine.—To that volume of the master solution which contains 0.2 gram of the dye add 1 c. c. of strong hydrochloric acid and bring to a volume of 50 c. c. Extract the lower sulphonated dye by shaking the solution successively in three separatory funnels, each containing 50 c. c. of amyl alcohol. Wash the amyl alcohol extracts by shaking successively with three 50 c. c. portions of approximately 0.25 N hydrochloric acid, passing each acid portion through the three funnels in the order used for the original solution. (One volume of strong hydrochloric acid, diluted with 50 volumes of water, yields a solution of about 0.25 N strength.) Dilute the amyl alcohol in each funnel with 100 c. c. portions of gasoline (specific gravity 0.65), and remove the lower sulphonated dye by washing with several 10 c. c. portions of water, passing each portion through the three funnels in an order the reverse of that previously followed.

Determine the dye in the water extract by titration against standard titanium trichloride solution (p. 24), using 15 grams of sodium acid tartrate and having a volume of 100 c. c. Run a blank determination on the same weight of tartrate and the same volume of water, using 1 mg. of the dye. Calculate the result to percentage of fast red E in amaranth and fast wool yellow G in tartrazine. One cubic centimeter of 0.1 N titanium trichloride solution is equivalent to 0.01256 gram of fast red E and to 0.01081 gram of fast wool yellow G. If the quantity of dye is very small, it may be determined colorimetrically, using the original dye as a standard.

For ponceau 3R.—Proceed as directed in the first paragraphs of this section, but use a mixture of equal volumes of amyl alcohol and gasoline (specific gravity 0.65) in place of the amyl alcohol and run the blank with 1 mg. of ponceau 3R. Calculate the result as percentage of sodium trimethylbenzene-azo-β-naphtholsulphonate, using the factor 0.009807.

For indigotine.—Proceed as directed in the first paragraphs of this section, but use 0.25 c. c. of acid instead of 1 c. c., wash with sixteenth normal instead of 0.25 N acid, and run the blank with 1 mg. of indigotine. Calculate the result as percentage of sodium indigo monosulphonate. One cubic centimeter of 0.1 N titanium trichloride solution is equivalent to 0.01821 gram of sodium indigo monosulphonate.

For light green S F yellowish.—To a solution of 125 grams of sodium chloride in 400 c. c. of water add 12 c. c. of glacial acetic acid and a solution containing 13.6 grams of sodium acetate ($NaC_2H_3O_2.3H_2O$) and dilute to 500 c. c. To that volume of the master solution which contains 0.1 gram of the dye add water, if necessary, to bring to a volume of 10 c. c. Dilute with 40 c. c. of the salt-acetate solution and extract successively in three separatory funnels, each containing 100 c. c. of amyl alcohol. Wash the extracts with three 100 c. c. portions of the salt-acetate solution, passing each wash portion successively through the three funnels in the order followed in making the original extractions. Remove the dye from the alcohol as directed in the first paragraph of this section and determine colorimetrically by comparison with a standard guinea green B solution of approximately the same strength. Report as percentage of guinea green B.

BOILING POINT OF Ψ-CUMIDINE FROM PONCEAU 3R

Reduction and Extraction

Dissolve 60 grams of the dye in a 600 to 700 c. c. beaker with about 450 c. c. of boiling water and add it very slowly to a warm (60° to 80° C.) solution of 100 grams of stannous chloride in 100 c. c. of strong hydrochloric acid in a tall 1200 c. c. beaker. Add 10 to 20 c. c. of the dye solution at a time, waiting after each addition until the mixture is pale brown. Otherwise the dye will be precipitated, in which case it can be reduced only with difficulty. As reduction proceeds and the solution becomes more dilute heat to boiling, taking care that the mixture does not boil over after each addition of dye, as some heat is generated by the reaction. After all the dye has been added and reduced, let the mixture cool and make it alkaline by adding about 75 grams of sodium hydroxide dissolved in 150 to 200 c. c. of water.

Cool the alkaline mixture and shake it with three 200 c. c. portions of ether. Combine the ether extracts of ψ-cumidine thus obtained and wash with water until the alkali and salts are removed. Evaporate the solvent on the steam bath, but avoid such prolonged heating as may tend to volatilize the base. Transfer the residue of ψ-cumidine to a small side-neck flask and distil it, carefully avoiding overheating. Observe the range within which the substance volatilizes.

Reduction and Steam Distillation

Proceed as directed in the first paragraph under reduction and extraction. Then steam distil the alkaline mixture until no more oil is carried over. Separate the oil layer and extract the water layer with two 150 c. c. portions of ether. Add the extracts to the oil layer and wash the mixture with successive 10 c. c. portions of water until the alkali and salts are removed. Complete the determination as directed under reduction and extraction, beginning with "Evaporate the solvent on the steam bath."

ISOMERIC AND SIMILAR DYES IN AMARANTH

Take that volume of the master solution which contains 0.1 gram of the dye and dilute, if necessary, to 40 c. c. with water. Add 10 c. c. of 0.1 N benzidine solution (9.2 grams of base per liter in 0.5 N hydrochloric acid), mix well, and let stand exactly two minutes. Filter through a fluted paper and dilute 10 c. c. of the filtrate to 100 c. c. Compare this solution colorimetrically with a standard amaranth solution containing 0.4 mg. of the dye per 100 c. c. The master solution of the amaranth to be tested may be used in making the standard solution. If, after the benzidine treatment, the solution obtained is not more deeply colored than the standard solution, the proportion of isomeric dyes may be considered to be below 1.5 per cent.

SODIUM IODIDE

For erythrosine.—Dilute to about 400 c. c. that volume of the master solution which contains 5 grams of the dye and add a mixture of 2 c. c. of strong nitric acid and 10 to 20 c. c. of water. Dilute to exactly 500 c. c., mix, and filter through a dry paper. Place 200 c. c. of the filtrate in a porcelain casserole and make slightly alkaline with 10 per cent sodium hydroxide solution. Add about 20 c. c. of 7 per cent potassium permanganate, mix, and add 10 c. c. of strong nitric acid. Place on a steam bath and evaporate to dryness. Add 5 c. c. of 7 per cent potassium permanganate and 5 c. c. of strong nitric acid and again evaporate to dryness. Then add about 50 c. c. of water, 5 c. c. of strong nitric acid, and 25 to 30 c. c. of a saturated solution of sulphur dioxide (p. 15). Stir frequently, breaking up any lumps, until the hydrated oxide of manganese is dissolved. Filter, wash the paper with water, and add to the combined filtrate and washings an excess of 10 per cent silver nitrate solution. Boil until the sulphur dioxide has been expelled. When cool collect the silver iodide on a weighed Gooch crucible. Wash with hot water, dry, and weigh. Calculate as percentage of sodium iodide.

IODINE ORGANICALLY COMBINED

For erythrosine.—Place in a porcelain casserole that volume of the master solution which contains 0.3 to 0.4 gram of the dye. Add 5 c. c. of a 10 per cent sodium hydroxide solution and 35 c. c. of a 7 per cent solution of pure potassium permanganate and mix. Partially cover the vessel with a watch glass and add 10 c. c. of strong nitric acid. Place on a steam bath and keep covered until spattering ceases. Remove and wash the watch glass and allow evaporation to proceed

to dryness. (Take care to prevent the access of reducing gases or vapors to the mixture.) Treat the residue with 5 c. c. of 7 per cent potassium permanganate and 5 c. c. of strong nitric acid and again evaporate to dryness. Add about 50 c. c. of water and 5 c. c. of strong nitric acid to the residue. Then add 40 c. c. of a saturated solution of sulphur dioxide (p. 15), break up the lumps with a glass rod, and let stand, with occasional stirring, until the hydrated oxide of manganese is dissolved. Filter, wash the paper thoroughly with water, add an excess of 10 per cent silver nitrate solution to the combined filtrate and washings, and boil until sulphur dioxide has been expelled and the silver iodide has flocculated. Collect the precipitate on a weighed Gooch crucible, wash with hot water, dry, and weigh. Calculate as percentage of free iodine and from this subtract the percentage of iodine found as sodium iodide (p. 30).

TOTAL HALOGENS

For erythrosine.—Mix 0.5 to 1 gram of the dye with 4 grams of potassium carbonate and moisten to a paste with 50 per cent alcohol. Dry, cover with a layer of dry potassium carbonate, and ignite at a low red heat. Allow to cool, moisten with a few drops of water, and break up the charred mass thoroughly. Wash into a beaker and bring to a volume of about 200 c. c. with water, allow to digest for 15 minutes, and filter. Wash the insoluble matter until the washings no longer react with silver nitrate; then acidify the filtrate and washings with nitric acid, using an excess equivalent to 5 c. c. of the strong acid, and precipitate the halogens with 10 per cent silver nitrate solution. Collect the precipitate on a weighed Gooch crucible, wash, dry, and weigh. Compare with the sum of the results obtained in the separate halogen determinations.

SODIUM CARBONATE IN ERYTHROSINE

Apparatus

Arrange a train of apparatus as shown in Figure 4. A glass tube (*A*), with an internal diameter of about 15 mm. and filled as shown with solid glass beads, is inserted in a round-bottom 1000 c. c. flask (*B*). This flask is connected with a 600 c. c. flask (*E*), fitted with a perforated rubber stopper, through which pass the delivery tube of a short reflux condenser (*C*), the stem of a small dropping funnel (*D*), and a tube (*F*), leading to three 16-ounce bottles (*G*, *H*, and *I*). *G* is partly filled with carbon-dioxide-free water (about 300 c. c.) and glass beads; *H* and *I* contain about 300 c. c. of 50 per cent sodium hydroxide solution and glass beads.

A U-tube containing standard barium hydroxide solution may also be provided to show whether or not the apparatus has been freed from uncombined carbon dioxide and whether all the carbon dioxide in the sample has been taken up by the barium hydroxide in *B*. If a cloudiness develops in the U-tube at the end of the procedure, showing that the carbon dioxide has not been completely taken up, repeat the analysis, using a smaller sample of dye. When connection is made with the air pump, provide the rubber tubing used with two screw clamps or some other device that will permit the rate of the flow of air drawn through to be readily and accurately adjusted.

Determination

Connect the apparatus as shown in Figure 4 and place 10 grams of the dye, a few glass beads, and about 250 c. c. of carbon-dioxide-free water in the flask *E*. Pass cold water through the outer jacket of condenser *C*. Insert the stopper in the dropping funnel, attach the air pump at *K*, and draw air through the apparatus for about 20 minutes.

Toward the end of the 20-minute period, close the stopcock of the dropping funnel, pour into the dropping funnel a mixture of 4 c. c. of concentrated sulphuric acid and 10 to 15 c. c. of distilled water free from carbon dioxide, and reinsert the stopper. Disconnect suction and at *K* connect the U-tube containing the standard barium hydroxide. Reconnect the suction beyond the U-tube and draw air through the entire train for five minutes. If the barium hydroxide remains clear the train may be considered to be free from uncombined carbon dioxide.

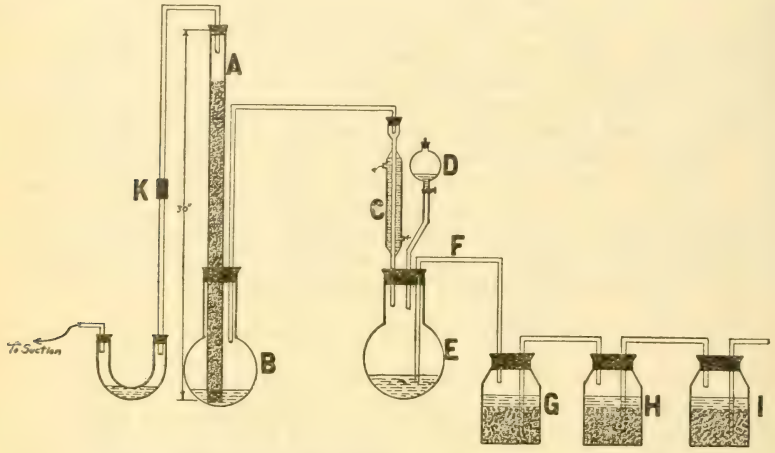

FIG. 4.—Apparatus for determination of sodium carbonate in erythrosine

Place 50 c. c. of standard approximately 0.1 N barium hydroxide in the glass tube *A*. Draw air through the entire train for a few minutes and then discontinue the suction. Open the stopcock and the stopper of the dropping funnel so that the acid may flow into the dye solution, taking care to close the stopcock immediately when only a few drops remain in the funnel. Reconnect the air pump, heat the dye solution rapidly to boiling, and continue to draw air through the apparatus, slowly at first, until all the carbon dioxide has been carried over into the standard barium hydroxide. When the evolution of carbon dioxide has ceased, disconnect the flask *B*, raise the tube *A*, and let the beads slide into the flask. Wash the tube quickly with a little carbon-dioxide-free water, close the flask with a stopper and bunsen valve or other device to prevent access of carbon dioxide, and heat to boiling to make the precipitate crystalline and less soluble. Finally cool and titrate carefully with standard hydrochloric acid, using phenolphthalein as an indicator. Calculate as percentage of sodium carbonate.

ORANGE II AND OTHER SUBSIDIARY DYES IN ORANGE I

To that volume of the master solution which contains 1 gram of dye add water, if necessary, to bring the volume to 100 c. c. and then 10 c. c. of strong hydrochloric acid. Extract this solution by shaking successively in three 500 c. c. separatory funnels, each containing 100 c. c. of amyl alcohol and 5 c. c. of strong hydrochloric acid. Wash each of the three amyl alcohol extracts by means of six 100 c. c. portions of normal sodium carbonate solution (53 grams of anhydrous sodium carbonate to the liter), passed successively through the funnels in the order first used. (In washing the acidified amyl alcohol solutions, shake gently at first, keeping the funnel upright and unstoppered until the evolution of carbon dioxide is slow enough to permit more vigorous shaking.) In the same manner wash the extracts in the second and third funnels with two more 100 c. c. portions of the sodium carbonate and wash the extract in the third funnel with two additional portions of the carbonate solution. Dilute the amyl alcohol solutions by adding 350 c. c. of gasoline (specific gravity 0.65) to each funnel. Remove the dye by extracting completely with the requisite number of 10 c. c. portions of water passed through the funnels, reversing the order previously used. Bring the volume to 150 c. c. by adding water, add about 10 grams of sodium acid tartrate, and titrate under carbon dioxide with standard titanium trichloride solution (p. 24). Run a blank, using 10 grams of sodium acid tartrate and 150 c. c. of water, with 1 mg. of orange I for an indicator. Calculate as percentage of orange II, 1 c. c. of 0.1 N titanium trichloride being equivalent to 0.008756 gram of the dye.

MARTIUS YELLOW IN NAPHTHOL YELLOW S

Dissolve 5 grams of the dye in 150 c. c. of water, add 5 c. c. of strong hydrochloric acid, and shake vigorously in a separatory funnel for one minute with 50 c. c. of gasoline (specific gravity 0.65). Separate the solutions and extract the aqueous liquid again with 25 to 30 c. c. of the solvent. Combine the portions of gasoline, decant into a clean separatory funnel, and wash with four 25 c. c. portions of 0.25 N hydrochloric acid. Shake with a few portions of 5 per cent sodium hydroxide solution to extract the martius yellow. Neutralize the alkaline dye solution with tartaric acid, add 5 grams of sodium tartrate, and titrate against standard titanium trichloride as directed on page 26. One cubic centimeter of 0.1 N titanium trichloride is equivalent to 0.002134 gram of martius yellow.

Very small quantities may also be estimated colorimetrically (in neutral or slightly alkaline solution) by comparison with a standard naphthol yellow S solution, the tinctorial power of which is considered to be eight-tenths that of martius yellow. It is calculated as percentage of martius yellow.

TOTAL NITROGEN

For ponceau 3R, amaranth, orange I, naphthol yellow S, tartrazine, yellow A B, yellow O B, guinea green B, light green S F yellowish, and indigotine.—Use the method of Dumas (*17*).

For guinea green B, light green S F yellowish, and indigotine.—Determine on 2-gram portions by Gunning's modification of the Kjeldahl process (*44*). Use a little copper sulphate to assist the oxidation.

For ponceau 3R, amaranth, orange I, and tartrazine.—Treat 2 grams of the dye with 25 c. c. of a saturated solution of sulphur dioxide (p. 15) and 1 gram of zinc dust, and warm the mixture gently until it becomes colorless. This should take place in from two to three minutes. If it does not, add more sulphur dioxide solution in small portions at a time until the color is destroyed. Then add 30 c. c. of concentrated sulphuric acid and 0.7 gram of mercuric oxide or its equivalent of metallic mercury and digest the mixture. Finally make alkaline and distil as usual in the Kjeldahl method.

TABLE 13.—*Percentage of sulphur, nitrogen, and sodium in permitted food dyes*

Dye	Sulphur	Nitrogen	Sodium	Sodium sulphate corresponding to sodium content
	Per cent	*Per cent*	*Per cent*	*Per cent*
Ponceau 3R	12.97	5.66	9.30	28.71
Amaranth	15.91	4.64	11.41	35.23
Erythrosine	----------	----------	5.12	15.81
Orange I	9.15	8.00	6.57	20.28
Naphthol yellow S	8.95	7.82	12.84	39.64
Tartrazine:				
Trisodium salt	12.00	10.49	12.91	39.85
Disodium salt	12.52	10.94	8.98	27.72
Guinea green B [1]	9.29	4.06	3.33	10.28
Light green S F yellowish: [1]				
Disodium salt	12.13	3.53	5.80	17.90
Monosodium salt	12.48	3.64	2.98	9.20
Indigotine	13.75	6.01	9.86	30.42
Yellow A B	----------	17.00	----------	----------
Yellow O B	----------	16.09	----------	----------

[1] Recently acquired evidence indicates that the disodium salt of guinea green B and the trisodium salt of light green S F yellowish form colorless solutions (*72*).

COLOR BY SPECTROPHOTOMETER

The accuracy of the spectrophotometer for the quantitative estimation of color depends upon the skill of the operator in distinguishing between slight changes in the intensity of monochromatic light. For this reason quantitative estimations should be undertaken only by those who are thoroughly familiar with this instrument.

Buffer Solutions

Prepare a buffer solution by one of the following methods:

(1) Dissolve 71.4 grams of disodium phosphate ($Na_2HPO_4.12H_2O$) and 9.6 c. c. of glacial acetic acid in water and dilute to 1 liter. For use, dilute 5 c. c. with 95 c. c. of water.

(2) Dissolve 3.540 grams of primary potassium phosphate (KH_2PO_4) and 14.568 grams of disodium phosphate ($Na_2HPO_4.12H_2O$) in water and dilute to 1 liter. For use, dilute with an equal volume of water.

Preparation of Sample

All water-soluble dyes.—Dissolve 10 mg. of dye in 500 c. c. of the diluted buffer.

Yellow A B and yellow O B.—Dissolve 10 mg. of dye in 500 c. c. of alcohol (95 per cent).

Determination

Fill a 1 cm. cell with the dye solution prepared as directed and place it in front of one orifice of the spectrophotometer. Fill another cell of the same size with the solvent used and place it before the other orifice. The second cell corrects for any error which may be caused by the absorption of light by the solvent. Rotate the photometer control until the two fields appear matched and record the extinction coefficient. The most accurate results can usually be obtained by rotating the photometer control rapidly back and forth, making first one field and then the other the darker, and stopping in the middle of the swing. With a little experience the extinction coefficients can be determined to within 2 per cent. Record the extinction coefficients for the point of maximum absorption and at least one point on each side of the maximum. For yellow A B and yellow O B determine the extinction coefficients at 440 $\mu\mu$ and 450 $\mu\mu$.

The color content is found by dividing the observed extinction coefficients by the extinction coefficients found for a 0.002 per cent solution at the same wave length and dissolved in the same buffer solution.

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